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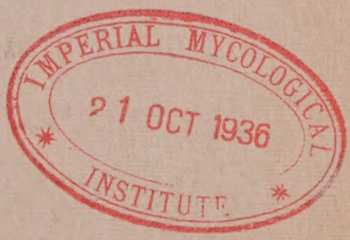

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SUMMARY

A survey of corn smut based on visible smut galls and conducted near Ames, Iowa, from 1930 to 1934 inclusive, showed 10.6, 9.9, 13.9, 5.5 and 18.3 percent of affected plants.

When the leaf sheaths were stripped from 1,985 plants, exposed to artificial and natural infection in 1934, many small smut galls aggregating 39.3 percent of the total expressed infections were found at the nodes.

The symptoms of corn smut may be classed as exposed and concealed. Exposed symptoms occur as irregular yellow or reddish stripes or blotches, brownish lesions and galls. Concealed symptoms are completely hidden by the leaf sheaths and occur as small nodal galls and minute pustules of chlamydospores in the leaves of the axillary buds.

A decrease in the surface tension of the inoculum increased the infectivity of the medium containing the sporidia. One percent rosin fish oil soap was found to be an effective surface tension reducing agent. The surface tension of carrot filtrate, used as a culture medium, was 47.0 dynes per centimeter; water, 72.3; carrot filtrare with 1 percent fish oil soap, 34.0.

Ninety-two percent of 500 sweet corn plants (Golden Bantam) became infected when inoculated with a sporidial suspension in carrot filtrate plus 1 percent fish oil soap, while 36.6 percent of 90 similar plants became infected when inoculated with a sporidial suspension in carrot filtrate.

Increased bud growth was accompanied by a larger number of nodal infections. Axillary bud growth was stimulated by injury to the corn plant, or inhibition of pollination. Injured or unpollinated inoculated plants produced approximately twice as many smut galls as the checks.

Histological examination of 262 axillary buds from 50 inoculated sweet corn plants (Golden Bantam) showed 53.6 percent or 140 of the buds to be infected with smut mycelium. Mycelium was found in apparently healthy axillary buds 67 days after inoculation.

The percentage of smut infection as indicated by nodal smut galls increased with lateness in planting. An average of data for 2 years showed 12 percent on May 15 plantings while approximately 40 percent of the June 4 plantings were smutted.

The percentage of smut infection as indicated by nodal smut galls showed a tendency to increase both ways from the 2-3 rates of planting. During the years 1931, 1932, 1933 and 1934 those plants growing one in a hill showed 40.8, 20.4, 9.0 and 22.8 percent infection, respectively; those at the rate of three per hill 22.5, 13.5, 4.3 and 14.6, while those at the rate of five per hill showed 32.4, 21.6, 5.9 and 18.5 percent infection.

Some of the Factors Influencing the Infection and Pathogenicity of *Ustilago Zeae* (Beckm.) Unger on *Zea Mays* L.[1]

By Glen N. Davis[2]

A half century has passed since Brefeld (2) in 1883 published his excellent monograph on the smuts. He used the following three methods of exposing the plants to infection: (1) infesting with sporidia the soil in which the corn was planted; (2) spraying the entire plant with a suspension of sporidia; and (3) dropping the sporidial suspension into the spiral whorl with a wash bottle.

Since Brefeld's time much has been learned regarding the corn smut organism and its host. Brefeld (2) and Hitchcock and Norton (14) established that corn plants may be infected by any method of inoculation whereby viable sporidia are brought into contact with meristematic tissue of the plant. Christensen and Stakman (4) have established that *Ustilago zeae* is a group species, including at least seven physiologic forms. Jones (20), Hayes, et al., (12), Garber (7), Garber and Quisenberry (8), and Immer and Christensen (16) have shown that self-fertilization of corn leads to the isolation of relatively homozygous strains which differ strikingly in their susceptibility to corn smut. Griffiths (9) has demonstrated that lines of corn showing resistance to natural infection in the field are susceptible when inoculated in the greenhouse by injecting a sporidial suspension into the young tissue. It has been shown by Platz (23) that the axillary buds of corn plants do not become infected when inoculum is dropped behind the leaf sheaths, and in more recent years evidence has been presented by Stakman and Christensen (26), Hanna (11), Christensen (3), Sleumer (25), Bauch (1) and Seyfert (24) showing that *Ustilago zeae* is primarily heterothallic with the occasional occurrence of a homothallic or "solopathogenic" monosporidial strain.

However important the foregoing contributions may be, very little has been learned regarding the true nature of resistance

[1] Taken from a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree, doctor of philosophy. Project No. 92 of the Iowa Agricultural Experiment Station.

[2] The author expresses his sincere appreciation to Dr. I. E. Melhus for his assistance and helpful advice in direction of the work and preparation of the manuscript.

to corn smut and the interrelationship between the pathogen and the host. No new contributions have been made to the methods of infection since the development of Brefeld's (2) "spritzflasche" method in 1883 and the method of injecting sporidia into wounds, developed by Hitchcock and Norton (14) in 1896. Practically nothing is known regarding the production and function of "air conidia" in nature, and knowledge relating to the ecological relations of the host and pathogen is equally limited. Likewise, information regarding the control of corn smut is limited to a few unsuccessful attempts with dusting or spraying practices and the recommendation of nonspecific cultural practices.

In this paper it is proposed to give consideration to certain of the problems mentioned above. In undertaking the problem of infection, however, it was soon apparent that the known methods of inoculation were not well suited to a study of infection phenomena. Therefore, ways of insuring a higher percentage of infection when plants were inoculated were considered. The results obtained soon showed that development of the host had a marked influence on the development of the pathogen after infection had actually taken place.

SYMPTOMS

Corn smut symptoms appear on the various aerial parts of the corn plant, anywhere from the tip of the tassel to the ground line and include chlorophyll changes such as blotches, striping and the formation of anthocyan pigment, necrosis and hyperplasia of infected organs.

CHLOROPHYLL CHANGES

One of the earliest symptoms to appear, and one of the symptoms of corn smut most frequently overlooked, is yellowing of the leaves owing to a reduction in the amount of chlorophyll in localized infected areas. Many times leaf infection, due to maturation of the tissues, does not proceed to the production of smut galls but remains throughout the life of the plant as yellowish irregular areas or streaks. Yellowing may occur at any point from the tip to the base of the leaf blade, and infected areas may range in size from minute streaks between the vascular bundles to elongated streaks extending practically the entire length of the leaf. Infected areas may also occur in irregular shapes which do not seem to be limited by the vascular bundles and, like streaking, may be limited to

minute spots or in some cases may include the entire leaf. Such symptoms are easily located on young plants but become increasingly difficult to identify as the corn plant matures. In some cases they may entirely disappear.

Infected areas may appear on the leaves of corn plants as reddish or purple irregular blotches or streaks. Like yellowing, the discolored areas may appear anywhere on the leaf blade and vary in size from minute spots to larger regions covering most of the leaf. The red or purple areas may appear in solid spots or they may completely encircle yellowed areas or regions of gall production. When portions of a leaf bearing these discolored areas are examined under the microscope it is seen that many of the epidermal cells contain a red coloring matter in solution in the cell sap. Occasionally this red substance separates out in crystals. Hanna (11) has shown that the pigment involved is anthocyan which accumulates in the epidermal cells of the infected areas and may produce an intense coloration. This latter action is dependent, seemingly, upon the varietal response of the host plant and environmental conditions.

GALLS

The symptom most easily recognized and undoubtedly the one upon which most smut readings are based is the formation of galls due to hypertrophy of the infected tissue. Smut galls may vary in size from minute pustules on the leaves to others several inches in diameter on the stalks or ears. They are at first of a whitish grey color owing to a covering membrane of host tissue, later turning black with the development of the enclosed smut mass; upon reaching maturity the covering membrane dries and breaks, exposing the dry powdery mass of spores. Smut galls may appear at almost any place where meristimatic tissue occurs, but they are common on or near the midrib of the leaves, at the junction of the leaf sheath and blade or at the nodal buds on the stems. Individual flowers of the ear, groups of flowers or the entire ear may be involved. In the staminate inflorescence the individual organs may be converted into smut galls. Many small smut galls, which are commonly overlooked, occur at the nodes below the ear of the corn plant. They are formed in the tissue of the axillary buds and consist of masses of spores varying in size from those barely recognizable with the unaided eye to elongated flattened galls several inches in length. Often, when the pressure of the galls is not sufficient to rupture the leaf sheath, they are almost completely enclosed and hidden by the leaf sheath and may be determined only by stripping the leaves from the corn plants.

NECROSIS

Where the pathogene has invaded the leaves the parenchymatous tissue between the fibro-vascular bundles is frequently killed. The areas involved may be only small circular spots or long linear strip-like areas. Frequently in the larger areas the dead tissue breaks away leaving an opening in the leaf. Such symptoms persist throughout the life of the corn plant, but in the case of older plants may be easily confused with shredding as the result of other causes. Often infections which do not develop sufficiently for gall formation occur in the leaves of the axillary buds, and may, upon careful examination, appear as small necrotic areas of the individual leaves of the latent axillary buds.

PREVALENCE AND DISTRIBUTION

Corn smut is world-wide in its distribution, but its destructiveness varies from one section to another and from year to year and may be interpreted in terms of prevalence and distribution.

Data made available by the United States Department of Agriculture Plant Disease Reporter for 1928, 1929, 1930 and 1931 show that 1928 losses were greater than average from Ohio westward to Kansas and in the dry farming regions of Colorado, California and Arizona. Estimated losses in 1928 ranged from 15 percent in California to 0.3 percent in Maryland, while the estimated loss for the whole United States was 4 percent. The prevalence of corn smut in 1929 was considered to be about average, (3.5 to 5 percent), with an unusual amount of ear infection reported from Connecticut, Pennsylvania, Ohio and Iowa. In Mississippi and Arkansas corn smut was noted as being especially prevalent in some of the overflowed river valleys where corn was grown as a late crop. In the same year reported infection ranged from 5 percent in Ohio, Nebraska and Iowa to a trace in states west of the Rocky Mountains, while the estimated loss in yield for the United States was 3.2 percent. In 1930 reported infection varied from 5 percent in West Virginia, Iowa and Nebraska to a trace in western states, causing an estimated loss of 2 percent. Data presented for the year 1933 show corn smut as unusually prevalent in Massachusetts and some of the Great Lakes states, the greatest damage occurring in sweet corn. Estimated losses ranged from 15 percent in sweet corn in Michigan to a trace in some of the less important corn growing regions. The estimated loss in field corn for the United States in 1933 was approximately 3 percent.

Immer and Christensen (17) (18) and Johnson and Christensen (19) have investigated the losses caused by corn smut infection. Their study was made on pairs of plants of the same selfed lines of corn growing 1 foot apart and subjected to the same amount of competition from surrounding plants. Their investigations indicate that the average reduction in yield caused by smut infection, irrespective of size or location of smut galls, was approximately 25 percent for single galls and about 50 percent for multiple galls. On the basis of the percentage of plants with multiple and single galls, the average reduction in yield per plant due to smut is estimated to be approximately 30 percent. They believe that losses due to corn smut have likely been underestimated and their results emphasize the need of breeding for smut resistance in corn.

Data presented by Platz (23) on smut infection of Iodent, a strain of Reid's Yellow Dent corn, near Ames, Iowa, for the years 1923 and 1927 show that corn smut was more prevalent during 1927 with an average of 15.7 percent infection as compared with 7.9 percent for 1923. Of 627 plants infected in 1927, 307 showed nodal infection. Most of the smut was limited to individual or specific parts of the plants. In 67.1 percent of 627 diseased plants only one part of the plant was attacked; in 32.9 percent of the plants two or more parts of the stalk were infected, and in 1.8 percent the entire top of the plant was attacked.

A smut survey was conducted in several fields near Ames, Iowa, at the end of the growing seasons of 1930 to 1934, inclusive (table 1). All readings were based on visible smut galls regardless of their location on the plant. Table 1 shows that of the 2,924 plants observed in 1930, 312 or 10.6 percent produced smut galls, 90 percent of which occurred as nodal infections; the remaining 10 percent were distributed over the leaves and tassels. During the summer of 1930 smut galls appeared on 8.9 percent of the plants with approximately the same ratio between leaf, tassel and nodal infection as was found in 1929. Smut galls appeared on 13.9 percent of 2,303 observed plants

TABLE 1. NUMBER AND PERCENTAGE OF PLANTS INFECTED WITH SMUT GALLS IN FIELDS NEAR AMES, IOWA, IN 1930, 1931, 1932 AND 1933.

Year	Number plants	Number plants infected	Percentage plants infected
1930	2,924	312	10.6
1931	1,700	152	8.9
1932	2,303	321	13.9
1933	1,000	55	5.5
1934	1,200	275	18.3

in 1932. Approximately 25 percent of the galls, however, appeared on the leaves and tassels, the remaining 75 percent occurring as nodal infections. The percentage of plants on which smut galls appeared during the summer of 1933 was the lowest of the 4 years recorded, with galls appearing on 5.5 percent of the observed plants. Ninety-five percent, however, were recorded as nodal infections with 5 percent appearing on the leaves and tassels. In 1934, 18.3 percent of 1,200 plants examined in fields near Ames, Iowa, were infected with corn smut. Eighty-eight percent of the total infection consisted of nodal smut galls. The remaining 12 percent included all other types of exposed infection.

When all the leaves were stripped from 240 plants (Reid's Yellow Dent) on Sept. 19, 1930, 101 nodal smut galls were found distributed on 72 (30 percent) of the 240 plants. A comparison with the smut reading for 1930 given in table 1 shows the amount of nodal smut infection exposed by stripping the plants to be approximately three times that determined without stripping the plants. Likewise in 1934, when the leaves were stripped from 1,985 plants exposed to natural and artificial infection, many small smut galls and necrotic lesions, aggregating 39.3 percent of the total infection, were found at the nodes. The foregoing data show that smut readings based on exposed symptoms alone are inaccurate and much too low. Similarly smut resistance or susceptibility of lines of corn based on anything less than a thorough examination of the corn plant for symptoms of infection may be inaccurate.

THE EFFECT OF CHANGES IN THE INOCULUM ON INFECTION

In the infection experiments with the corn smut organism here reported, the sporidia were placed in the spiral whorl of the plant with a pipette or injected into the growing point by the hypodermic injection method. During some of the inoculation experiments conducted in the fall of 1929 it was observed that when the sporidial suspension was dropped into the spiral whorls of the corn plants it did not immediately run down the center of the plants; due to some influence, probably surface tension, it seemed to remain as a large drop in the spiral whorl.

A macroscopic examination of the spiral whorl of a corn plant about 1½ feet tall showed it to be a funnel shaped opening, the sides of which are formed by the enveloping leaves of the corn plant. The lower end of the spiral whorl seems to be entirely closed by the small tightly wrapped leaves. This leaves no direct opening to the apical meristem; accessibility to

which is seemingly determined by the degree of tightness with which the young leaves are enveloped within one another. A microscopic examination of the upper surface of the corn leaves, which compose the spiral whorl, showed the epidermis to be covered with a shiny waxy appearing layer and an abundance of very tiny trichomes 80 to 100 per square centimeter and 750 to 800μ in length. When cross sections of the leaf were immersed in Sudan III for 1 hour and examined under the microscope, the covering layer had asssumed a pink color, giving the characteristic test for cutin. Cross sections of the leaves treated with ether yielded small irregularly shaped crystals of wax upon evaporation of the ether. The results of the above tests indicate that the corn leaves are covered by a protective layer of a fatty or waxy consistency. Such a surface seems to be extremely resistant to wetting, and only a solution of comparatively low surface tension does actually wet the leaves.

As a result of the foregoing examination of the spiral whorl of the corn plant, a series of experiments was planned to determine the effect upon the percentage of infection when plants were inoculated with sporidial suspensions to which various surface tension depressants had been added. Monosporidial cultures were used throughout the following experiments and were isolated in the following manner: A number of smut galls were collected from the field, and from these single chlamydospores were isolated by the dry needle manner described by Hanna (10). Each chlamydospore was placed in the center of a petri dish containing 2 percent potato dextrose agar. From these individual chlamydospores, monosporidial cultures were isolated with the Chamber's micro-manipulator. Each group of four cultures isolated from the same chlamydospore was lettered A, B, C, etc. Each monosporidial culture within a group of four was further identified by the numeral 1, 2, 3 or 4, depending on the cell of the promycelium from which it had been isolated. Culture No. 1 came from the apical cell of the promycelium while No. 4 came from the basal cell or the cell nearest the chlamydospore. Sexual compatability was determined by the method employed by Hanna (11).

Carrot filtrate in which the sporidial suspensions were grown was prepared by boiling 200 gms. of carrots in 1000 cc. of distilled water for ½ hour. All solid matter was removed by straining the solution through cloth. The resulting filtrate was sterilized at 20 pounds pressure for 15 minutes. Six to ten days preceding inoculation smut cultures were transferred from solid media into the carrot filtrate. The monosporidial cultures were grown in separate erlenmeyer flasks and mixed

TABLE 2. SURFACE TENSION AT 22°C. OF THE SUSPENSIONS USED IN INOCULATION EXPERIMENTS.

Suspension	Dynes per cm.
Distilled water	72.3
Carrot filtrate	47.0
0.5 percent acetone-carrot filtrate	43.6
1.0 percent acetone-carrot filtrate	41.24
0.5 percent fish oil soap-carrot filtrate	37.4
1.0 percent fish oil soap-carrot filtrate	34.04

just before inoculation by pouring the contents of one flask into the other and shaking thoroughly.

Glycerol, sodium oleate, acetone and fish oil soap were each added to the carrot filtrate to reduce the surface tension. As fish oil soap and acetone gave the lowest surface tension other substances were discarded. Surface tension was determined by use of the du Noüy tensiometer.

In table 2 may be seen the relative surface tension of the various combinations used in the following experiments. Distilled water, while not used as an inoculum, is included for comparison. The surface tension of water was 25.03 dynes higher than that of carrot filtrate and the ½ and 1 percent acetone and fish oil soap reduced the surface tension when mixed with carrot filtrate. The 1 percent fish oil soap seemed to be the most effective; it reduced the surface tension to 34.04 dynes per centimeter. If surface tension is a factor in preventing the inoculum from penetrating the spiral whorl and reaching the growing points of the corn plant, any one of the above combinations should give a higher percentage of infected plants, when used as an inoculum, than distilled water or carrot filtrate when used alone. The 1 percent fish oil soap carrot filtrate with smut sporidia in suspension will be referred to hereafter as "treated inoculum."

In order to determine how far the "treated inoculum" would penetrate the spiral whorls of corn plants three series of 10 corn plants 30 days old were inoculated by the pipette method with suspensions containing safranin dye. One series was inoculated with distilled water, one with carrot filtrate and one with "treated inoculum." Forty-eight hours after inoculation the plants were cut longitudinally to determine the distance to which each inoculum had penetrated the spiral whorls of the plants. The average distance from the growing tip of the inoculated plants to the point of inoculation was 13 centimeters. Actual measurement of penetration as indicated by the presence of the dye showed that distilled water penetrated an average of 2 centimeters, carrot filtrate an average of

4 centimeters, while the "treated inoculum" had reached the growing tip in every case, a distance of 13 centimeters. This indicates that an inoculum of low surface tension is able to penetrate the tightly enveloped leaves of the spiral whorl and carry the smut sporidia into contact with the growing points of the plant.

To determine the effect of the "treated inoculum" upon the pathogen, smut sporidia were transferred to two sets of erlenmeyer flasks, one set containing carrot filtrate and one containing "treated inoculum." After 1 week 1 cc. of each suspension was injected into a series of corn plants with a hypodermic needle. In every case smut galls were produced, indicating that the sporidia were uninjured.

SOAP INJURY TO CORN PLANTS

To determine the effect upon the corn plants several cc. of each medium were dropped into the spiral whorls of a series of corn plants 35 days old. Two, 4 and 7 days later freehand sections were made of the plants. No injury, as indicated by necrosis, discoloration or distortion of the tissue could be detected. It was impossible, however, to determine whether or not minute injuries might result from the media in question. It has been observed that the "treated inoculum," when used during periods of excessively high temperatures, causes the leaves comprising the spiral whorl to stick together to varying degrees, preventing their natural unfolding. The relation between such injury and the resulting percentage of infection has not been determined. Calculations were made to determine the quantity of fish oil soap which would come in contact with the plant tissue at any given point. On the basis of dropping 1 cc. of inoculum into the spiral whorl of each plant at the time of inoculation it was found that 0.01 gm. of fish oil soap would be placed in the spiral whorl of each plant. Actual measurement of the seven innermost leaves of the plant taken from the growing tip to a point 13 cm. above gave an average of 388 sq. cm. over which the inoculum containing 0.01 gm. of fish oil soap would be distributed. On this basis only 0.00000026 gm. of fish oil soap would be in contact with each square millimeter of leaf surface. Thus the amount coming in contact with the surface of the leaf at any given point would be infinitesimal.

INFECTION EXPERIMENTS

Three methods of infection were used in the following experiments. The first method was the one employed by Brefeld (2) and consists of dropping a sporidial suspension into the spiral whorl of the corn plant with a pipette or "spritzflasche." The

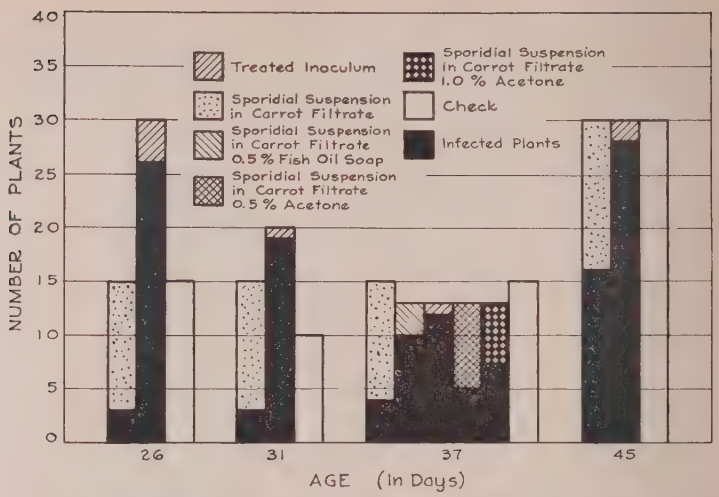

Fig. 1. Results of greenhouse inoculation experiments on sweet corn plants (Golden Bantam) in which sporidial suspensions of various surface tensions were employed as inoculum. The surface tensions of the sporidial suspensions in the various combinations were as follows: Carrot filtrate 47.0 dynes per sq. cm., one-half percent acetone carrot filtrate 43.6 dynes, one percent acetone carrot filtrate 41.24 dynes, one-half percent fish oil soap-carrot filtrate 37.4 dynes and one percent fish oil soap carrot filtrate (treated inoculum) 34.04 dynes. It should be noted that as the surface tension of the inoculum decreased the percentage of infected plants increased.

second method employed, the hypodermic injection, was probably first used in a modified form by Hitchcock and Norton (14) in 1896. The third or "treated inoculum" method, the one developed during this investigation, is the one upon which particular emphasis is placed in the following presentation of data. The "treated inoculum" method is primarily the one employed by Brefeld (2) except that the inoculum is modified through the addition of 1.0 percent fish oil soap just preceding inoculation.

The data presented in fig. 1 show the results obtained by inoculating four series of plants at the ages of 26, 31, 37 and 45 days, respectively. Part of the plants of each series was inoculated by each of the three methods. Because all results obtained by the hypodermic needle method of inoculation were the same, namely 100 percent infected plants in every case, they have been omitted in order to simplify the picture. By referring to fig. 1 it may be noted that when the pipette method of inoculation was used, decreased surface tension of the inoculum was accompanied by an increase in the amount of infection, with the "treated inoculum" showing a decided superiority in every case.

A further series of experiments is recorded in fig. 2. Figure 2 shows that when 90 sweet corn plants (Golden Bantam) were inoculated in the greenhouse with an inoculum of 47 dynes surface tension, 33 or 36.6 percent became infected; when 500 similar plants were inoculated with "treated inoculum" having a surface tension of 34 dynes, 460 or 92 percent became infected while 200 check plants remained free from infection. The results obtained in the field inoculation experiments and presented in fig. 2, while not so outstanding, are of similar trend.

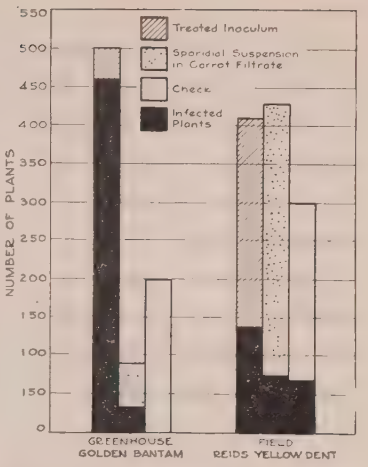

Fig. 2. A summary of greenhouse and field inoculation experiments with sporidial suspensions of varying surface tension. It should be noted that the "treated inoculum" with a surface tension of 34.0 dynes gave a higher percentage of infected plants than did the sporidial suspension in carrot filtrate, having a surface tension of 47.0 dynes per cm.

The results obtained by inoculating five lines of field corn of varying resistance to smut infection under field conditions are given in table 3. All the plants were inoculated by dropping 1 cc. of "treated inoculum" into the spiral whorls with a sterile pipette. All inoculations were made in the greenhouse. Three lines of corn were highly resistant to smut infection in the field, one was highly susceptible and one was intermediate in its reaction. In every case the percentage of infection was ap-

TABLE 3. PERCENTAGE OF SMUT INFECTION OBTAINED BY THE "TREATED INOCULUM" METHOD OF INOCULATION ON FIVE LINES OF CORN HAVING DIFFERENT REACTIONS TO NATURAL SMUT INFECTION.

Corn inbred*	Percentage natural infection	Number plants	Percentage plants smutted
309 by 361	1.7	21	33.3
303 by 360	0.0	20	30.0
302 by 360	2.1	70	35.0
309 by 357	30.8	21	52.4
805	60.0	41	90.0

*The inbred lines of corn used in the above experiment were supplied by Dr. M. T. Jenkins of the office of Cereal and Disease Investigations, United States Department of Agriculture and Farm Crops Subsection of the Iowa State Agricultural Experiment Station.

proximately 30 percent above that resulting from natural field infection. Likewise, in every case the plants maintained their position in respect to resistance or susceptibility; line 303x360 being the most resistant in both the field and in the greenhouse; line 805 being the most susceptible. It is possible that if an inoculum having a lower surface tension were available a still higher percentage of smut infection might be obtained by the pipette method of inoculation.

A brief review of the foregoing results shows that when sporidia were brought into direct contact with the meristematic tissue of the growing point of the corn plant by hypodermic injection 100 percent infection was practically assured. On the other hand, when plants were inoculated by the pipette method with a sporidial suspension in carrot filtrate a low percentage of infection was obtained, while plants inoculated with "treated inoculum" gave results more nearly like those obtained by the hypodermic injection method. Because the primary difference between the suspensions used for inoculum by the pipette method was a difference in surface tension, the higher percentages of infection by the "treated inoculum" method of inoculation may be attributed to the lower surface tension and the ability of the "treated inoculum" to carry the sporidia down through the spiral whorl where they may come into direct contact with the meristematic growing points of the corn plant.

PARASITISM OF *USTILAGO ZEAE* AS INFLUENCED BY ITS HOST DEVELOPMENT

It has been observed that nodal and ear infections are prevalent some years and rare others, and that such infections vary considerably in neighboring fields and in different sections of the same field. It has also been observed that smut galls appear at the nodes of corn plants growing in the field, beginning about July 20 and continuing until frost. It is a rather general opinion that smut galls on corn plants are the result of local infections which have taken place slightly prior to the time of expression and at the point of origin. If this is true, all nodal smut galls result from infection of the axillary bud at the point where the smut gall originates. Results obtained and set forth in the following presentation of data, however, have led to the belief that such is not the case. Rather, these data indicate that the corn smut organism infects the plant at an early stage of development and that its subsequent parasitism and expression are dependent upon the development of its host plant.

TABLE 4. NUMBER OF NODAL SMUT GALLS ON 90 SWEET CORN PLANTS (GOLDEN BANTAM) AFTER INOCULATION WITH USTILAGO ZEAE (BECKM.) UNGER.

Number plants	Date inoculation	Days after inoculation	Number nodal smut galls
90	December 6, 1931	30	6
		32	8
		35	12
		37	18
		39	24
		43	30
		45	32
		54	38
20	Checks		0

APPEARANCE OF NODAL SMUT GALLS

During the winter of 1932 a series of sweet corn plants (Golden Bantam) was inoculated when 25 days old. Inoculation was made by dropping 1 cc. of "treated inoculum" into the spiral whorl of each plant. The first reading for nodal smut galls was taken 30 days after inoculation and readings were continued during the next 54 days. The results given in table 4 show that 30 days after inoculation there were only six smut galls on the 90 plants; 32 days after there were eight. The number continued to increase with time until 54 days after inoculation, when there were 38. Because of the fact that 20 check plants remained free from smut infection every smut gall must have resulted from an infection which took place at the time of inoculation. This being the case, the mycelium must have remained in the axillary buds, while they were lifted high above their original position by the elongation of the internodes, and only expressed its presence by the development of a smut gall when the infected bud began to grow.

EFFECT OF INCREASED AXILLARY BUD GROWTH ON EXPRESSION OF NODAL SMUT INFECTION

STIMULATION OF AXILLARY BUD GROWTH BY INJURY TO THE CORN PLANT

Results obtained in the foregoing experiment suggested that infection may be present in the axillary buds of the corn plant, but may not be expressed until the buds develop, or in many cases where the bud fails to develop may never be expressed. The above suggestion led to the following experiments. During the summers of 1931, 1932, 1933 and 1934 field corn (Reid's Yellow Dent) was planted on or near May 10, and inoculated when about 1 foot tall by dropping 1½ to 2 cc. of

TABLE 5. NUMBERS AND PERCENTAGES OF PLANTS AND NODES INFECTED IN INJURED AND UNINJURED INOCULATED AND NON-INOCULATED PLANTS IN 1931

Treatment	Number plants	Method of injury Aug. 22	Number plants infected		Percentage of plants infected		Percentage increase in infection	Number nodal smut galls	
			Aug. 22	Sept. 17	Aug. 22	Sept. 17		Aug. 22	Sept. 17
Non-inoculated	98	Tops removed	14	19	14.2	19.3	5.1	16	23
" "	101	Ears removed	15	24	14.8	23.7	8.9	16	25
" "	101	Tops and ears removed	15	22	14.8	21.7	6.9	19	26
" "	103	Checks	14	19	13.5	18.4	4.9	15	20
Inoculated	202	Tops removed	41	44	20.3	26.7	6.4	44	57
"	205	Ears removed	32	49	15.6	23.4	7.8	38	62
"	207	Tops and ears removed	42	59	20.2	28.5	8.3	44	67
"	212	Checks	32	42	15.1	19.8	4.7	39	47

TABLE 6. NUMBERS AND PERCENTAGES OF PLANTS AND NODES INFECTED IN INJURED AND UNINJURED INOCULATED AND NON-INOCULATED PLANTS IN 1932.

Treatment	Number plants	Method of injury Aug. 16	Number plants infected		Percentage of plants infected		Percentage increase in infection	Number nodal smut galls	
			Aug. 16	Sept. 15	Aug. 16	Sept. 15		Aug. 16	Sept. 15
Non-inoculated	244	Tops removed	20	34	8.1	13.9	5.8	20	34
" "	214	Ears removed	11	27	5.1	12.6	7.5	12	30
" "	250	Tops and ears removed	26	43	10.4	17.1	6.7	26	50
" "	227	Checks	19	32	8.3	14.1	5.8	22	37
Inoculated	217	Tops removed	47	66	21.6	30.4	8.8	56	83
"	236	Ears removed	51	72	21.6	30.5	8.9	60	85
"	217	Tops and ears removed	39	59	18.0	27.1	9.1	41	65
"	225	Checks	61	72	27.1	32.0	4.9	75	86

TABLE 7. NUMBERS AND PERCENTAGES OF PLANTS AND NODES INFECTED IN INJURED AND UNINJURED INOCULATED AND NON-INOCULATED PLANTS IN 1933.

Treatment	Number plants	Method of injury Aug. 14	Number plants infected		Percentage of plants infected		Percentage increase in infection	Number nodal smut galls	
			Aug. 14	Sept. 22	Aug. 14	Sept. 22		Aug. 14	Sept. 22
Non-inoculated	169	Tops removed	5	10	2.9	5.9	3.0	3	8
" "	171	Ears removed	9	24	6.2	14.0	8.8	9	25
" "	172	Tops and ears removed	9	23	5.2	13.3	8.1	9	25
" "	178	Check	9	12	6.0	6.1	1.1	8	14
Inoculated	188	Tops removed	10	13	5.3	6.9	1.6	6	10
"	179	Ears removed	11	35	6.1	19.5	13.4	8	35
"	178	Tops and ears removed	15	27	8.4	15.1	6.7	14	29
"	171	Checks	8	15	4.7	8.8	4.1	7	14

"treated inoculum" into the spiral whorl of each plant. An equal number of non-inoculated plants was held as checks. About the middle of August smut readings were taken on all plants with special attention to nodal infection. Immediately after the August reading the plants were injured in three ways to increase axillary bud development and determine the effect of such increased bud development on the production of nodal smut galls. A series of inoculated and a series of non-inoculated plants were treated in the following ways: (1) Tops removed just above the ears, (2) ears removed, (3) tops and ears removed. Uninjured plants were held as checks. Final smut readings were taken about the middle of September.

Data obtained in 1931, 1932 and 1933 and set forth in tables 5, 6 and 7 show the number and percentage of inoculated and non-inoculated plants before and after injury and the number of nodal smut galls recorded for each treatment. While the individual treatments vary in regard to their significance when the data for any one year are considered, the same general trend is indicated throughout. When the data for the 3 years are combined, the increase in nodal smut galls from the August to the September reading among the inoculated plants is significantly greater in the injured plants than in the checks. There is also a significantly greater number of smut galls in the inoculated than in the non-inoculated series. On the other hand, when the increase in nodal smut galls from the August to the September reading in the injured and uninjured plants of the non-inoculated series is considered, the difference is not significant.

In the foregoing experiments the plants were all of the same variety, in the same field and under approximately similar growing conditions. Consequently the stimulation of bud development by injury to the plants should have been approximately the same in both the inoculated and non-inoculated plants, and the number of nodal smut galls should have been approximately equal if nodal galls result from local infection. As above noted, however, stimulated axillary bud activity in the inoculated series of plants was associated with a significant increase in the number of nodal smut galls expressed. Such was not the case in the non-inoculated series of plants, however, indicating that the increased infection must have taken place at the time of inoculation, early in the life of the plant.

A similar experiment recorded in table 8 was conducted during the summer of 1934. The removal of the ears, however, was the only method of injury employed. The 1934 season being exceedingly dry and hot and very unfavorable for the growing of corn, no significant increases in the expression of nodal infection were obtained. Table 8 is of interest, however, since in

TABLE 8. NUMBERS AND PERCENTAGES OF PLANTS AND NODES INFECTED IN INJURED AND UNINJURED INOCULATED AND UNINOCULATED PLANTS IN 1934.

Treatment	Number plants	Type of injury	Number plants infected			Percentage plants infected			Number nodal smut galls			Percentage nodal galls concealed by sheaths
			Sheaths present		Sheaths removed	Sheaths present		Sheaths removed	Sheaths present		Sheaths removed	
			Aug. 21	Sept. 27		Aug. 21	Sept. 27		Aug. 21	Sept. 27		
Non-inoculated	273	Checks	40	50	59	14.6	18.3	21.6	28	44	67	34.3
Non-inoculated	571	Ears removed	55	87	135	9.6	15.5	23.6	57	91	153	40.5
Inoculated	278	Checks	31	42	62	11.1	15.1	22.3	28	45	79	43.0
Inoculated	560	Ears removed	51	97	157	9.1	17.3	28.0	50	98	171	42.6

addition to the visibly expressed nodal infection upon which the data for 1931, 1932, 1933 and 1934 are based, with the exception of those columns headed "sheaths removed," a third reading taken after the removal of the leaf sheaths is recorded Davis (6)

Table 8 shows that in the non-inoculated checks 34.3 percent of the total nodal infection was concealed by the leaf sheaths while 40.5 percent of the total infection was obscured by the leaf sheaths in the injured non-inoculated series. In the inoculated uninjured series 43.0 percent and in the inoculated injured series 42.6 percent of the total nodal smut infection was concealed by the leaf sheaths.

As indicated by the foregoing results, many small smut galls which do not attain sufficient size to rupture the leaf sheaths may form in the tissues of the axillary buds. Such galls are necessarily overlooked unless the leaf sheaths are removed from the plants in question. This condition indicates the desirability of removing the leaf sheaths when exact smut readings are desired or when individual lines or varieties of corn are to be compared as to relative resistance to corn smut.

Figure 3 is a photograph of field corn plants free from smut infection. It shows quite characteristically the growth of axillary buds stimulated by the removal of the ear. It may be observed that every axillary bud remaining on the corn plant after removal of the ear has developed to some extent and remained free from smut infection. The upper axillary buds show a greater degree of development than those lower on the plant. Figure 4 is a photograph of stimulated axillary bud development on an inoculated plant, and while it represents a somewhat extreme case it shows the possibilities of infection and the necessity for the development of all the axillary buds

Fig. 3. Axillary bud growth on smut-free corn plants. Stimulation of axillary bud growth by removal of the ear caused the normal path of translocation to be disturbed and allowed the photosynthate generally stored in the ear to be distributed to the remaining axillary buds. While the buds nearest the apex of the corn plant show the greatest amount of growth it should be noted that all the axillary buds have grown to some extent.

Fig. 4. Stimulated axillary bud growth on a badly infected corn plant. Stimulation was accomplished by removing the ear. This figure shows that four of the lower axillary buds were infected with corn smut mycelium which did not express itself until the axillary buds in which it was located proceeded toward the formation of an ear.

on a corn plant to give a true picture of the total amount of smut infection.

From the foregoing data it is evident that stimulation of axillary bud development by injury to the corn plant was associated with an increase in the number of nodal smut galls produced. By removal of either the tops, the ears, or tops and ears, the normal path of translocation is disturbed and axillary bud growth stimulated. If increased axillary bud growth and increased nodal smut infection go hand in hand, as demonstrated by the foregoing experiments, one of two things must take place. The smut mycelium must be present in the axillary buds as the result of an earlier infection and only express itself when the axillary buds develop, or local infection takes place after the bud is stimulated to new growth and meristematic activity. The results obtained on the time of appearance of nodal smut galls in the greenhouse and field inoculation experiments reported in the foregoing tables, together with the negative results of Platz (23) in his attempts to cause nodal smut infection by inoculation behind the leaf sheaths, seem to justify the statement that nodal infection of the corn plant is the result of an infection early in the life of the corn plant. This infection becomes apparent when, for some reason, the axillary bud is stimulated to continued growth, allowing the organism to proceed to the development of a smut gall.

STIMULATION OF AXILLARY BUD GROWTH BY INHIBITION OF POLLINATION

To demonstrate further the presence of unexpressed infection in the axillary buds, and test still further the relationship of axillary bud growth and the production of nodal smut galls, the following experiments were conducted. Four rows of field corn (Reid's Yellow Dent) were planted on May 10, 1932. Two of the four rows were inoculated on June 10 by dropping about 1½ cc. of "treated inoculum" into the spiral whorl of each plant with a pipette. The plants were closely observed and a series of ear shoots on both inoculated and noninoculated plants were covered with paper sacks just before

TABLE 9. NUMBER AND PERCENTAGE OF SMUTTED PLANTS, WITH NUMBER OF NODAL SMUT GALLS IN FOUR TREATMENTS IN 1932.

Number plants	Treatment	Number plants smutted	Percentage plants smutted	Total number nodal smut galls
142	Plants inoculated, ears bagged	46	32.4	62
120	Plants inoculated, ears not bagged	14	11.6	14
120	Plants not inoculated, ears bagged	34	28.3	34
120	Plants not inoculated, ears not bagged	10	8.3	10

TABLE 10. NUMBER AND PERCENTAGE OF SMUTTED PLANTS, WITH NUMBER OF NODAL SMUT GALLS IN FOUR TREATMENTS IN 1933.

Number plants	Treatment	Number plants smutted	Percentage plants smutted	Total number nodal smut galls
174	Plants inoculated, ears bagged	38	21.8	42
262	Plants inoculated, ears not bagged	31	11.8	31
158	Plants not inoculated, ears bagged	22	13.9	22
274	Plants not inoculated, ears not bagged	17	6.2	17

silking. This prevented fertilization and stimulated axillary bud development. Often two or three ears were bagged on the same plant and on many stalks all axillary buds developed until several inches long. At the same time an equal number of stalks were tagged and allowed to develop normally as checks. About the time the silks appeared many of the plants were already visibly smutted. No plant showing any sign of smut infection at this time, however, was either bagged or tagged as a check. Therefore, all smut infections present at the final smut reading on Sept. 15 were expressed subsequent to bagging.

In the above experiments the results of which are shown in tables 9 and 10 all of the plants were of the same variety, growing in the same field in adjacent rows and under similar conditions. The stimulation of axillary bud development by bagging, therefore, should have been approximately the same in both inoculated and non-inoculated plants, and there should have been approximately the same number of nodal smut galls produced on the non-inoculated plants as on the inoculated plants if nodal smut galls are the result of local infection. This, however, was not the case. More plants in the inoculated series expressed nodal infection and they produced approximately double the number of smut galls that were produced in the non-inoculated series. This evidence further substantiates the contention that smut infection of corn takes place early in the life of the corn plant and nodal smut galls are produced only upon development of an axillary bud which is infected by smut mycelium. Therefore, at least half the buds which later developed into smut galls must have become infected at the time of inoculation by mycelium which did not proceed to gall formation until the development of the axillary bud. As shown by the results of bagging, in both inoculated and non-inoculated plants, buds are infected with smut mycelium which may never be expressed because the axillary bud in which it is present does not develop. This would have been the case had the ears not been bagged, which adds evidence of the close interrelation between host and parasite and the dependence of the parasite upon host development for its expression.

TABLE 11. NUMBER AND PERCENTAGE OF AXILLARY BUDS INFESTED IN INOCULATED SWEET CORN PLANTS (GOLDEN BANTAM).

Days after inoculation	Number plants	Number axillary buds examined	Number axillary buds infected	Percentage axillary buds infected
18	10	62	30	46.7
32	10	51	39	76.4
37	10	54	31	57.4
42	10	49	13	26.5
67	10	46	27	58.7
Summary	50	262	140	53.7

EXAMINATION OF AXILLARY BUDS FOR SMUT MYCELIUM

The results obtained in the foregoing experiments, indicating the presence of unexpressed infection and the close interrelation between host and parasite development, suggested the examination of axillary buds for the detection of smut mycelium to determine whether more buds were actually infected than indicated by the number of smut galls produced. A series of sweet corn plants (Golden Bantam) was planted in the greenhouse. When 30 days old they were inoculated by dropping 1 cc. of "treated inoculum" into the spiral whorl of each plant. Starting 18 days after inoculation and at intervals thereafter, 10 plants were selected at random and all axillary buds on all 10 plants examined for the presence of mycelium. If infection and its expression occur as shown by the foregoing data it should be possible to find mycelium of the smut organism in some of the axillary buds, which are, to all appearances, free from infection. Examination was made by cutting longitudinal free-hand sections from each bud. The

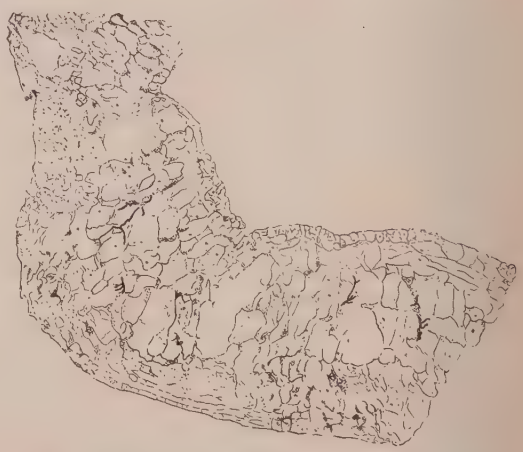

Fig. 5. Mycelium of the corn smut organism in the tissue of a 41-day-old axillary bud, sectioned and stained with Mayer's hemalum 14 days after inoculation. X50.

sections were stained with cotton blue in lactophenol and counter stained with eosin. The mycelium attained a deep blue and the plant tissue a light pink color. Histological sections and prepared slides were made to substantiate the determinations made by examination of the free-hand sections.

As shown by table 11, a total of 262 axillary buds from 50 plants was examined and of this number 140, or 53.7 percent, were found to be infected with smut mycelium. This number, 140 infected axillary buds, is greater than the number of smut galls produced on 50 plants. Evidently, therefore, a large percentage of the infected buds may never produce smut galls and the presence of smut mycelium in them is never expressed. These findings show that a corn plant may be susceptible to smut infection and actually may be infected but due to failure of the axillary buds to develop, no smut gall or other expression of the infection appears.

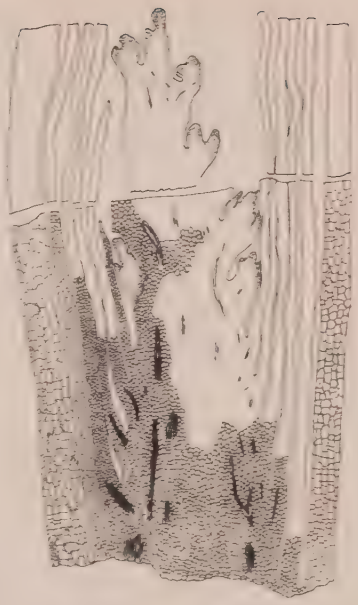

Fig. 6. Sketch of a 14-day-old axillary bud free from corn smut infection. It may be seen that the floral meristems have retained their identity and that there is no disorganization of the tissue. X50.

Figure 5 is a camera lucida drawing of a badly infected axillary bud, removed from the plant 14 days after inoculation. The small enveloping leaves were removed before the bud was sectioned and stained. An examination shows mycelial strands in three different regions of the bud. The tissue is badly hypertrophied, some of the cells having reached many times their original size. This stage of infection is easily discernible with the unaided eye. Sections of the infected tissue including the floral meristems have become so disorganized as completely to lose their identity. In contrast with this condition the sketch of a healthy axillary bud is shown in fig. 6. Here the floral meristems have retained their identity, and there is no hypertrophy or hyperplasia due to stimulation of the tissue by the corn smut organism.

Fig. 7. Camera lucida drawings of infected tissue taken from the tips of two apparently disease-free, 48-day-old axillary buds which were removed from an inoculated sweet corn plant (Golden Bantam) 18 days after inoculation. X700.

Fig. 8. Camera lucida drawing of smut mycelium in the tissue at the base of an apparently disease-free, 66-day-old axillary bud, removed from an inoculated sweet corn plant (Golden Bantam) 33 days after inoculation. X700.

Figures 7 to 10 are camera lucida drawings of mycelial strands in apparently healthy axillary buds. The buds were removed from inoculated sweet corn plants (Golden Bantam) at various intervals after the date of inoculation. The buds were killed, sectioned with a microtome and stained with Mayer's hemalum. The buds, sections of which are shown in fig. 7, were removed from the plants 18 days after inoculation, while those sections shown in figs. 8 and 9 are from axillary buds removed from the plant 33 days after inoculation. The section pictured in fig. 10 is from an axillary bud removed from the corn plant 67 days after the date of inoculation. The sections of plant tissue represented in figs. 7 to 10 were taken from axillary buds which were to all macroscopic appearances free from smut infection. An examination of the aforementioned figures shows that to all appearances no hypertrophy or hyperplasia has taken place although the bud tissue is penetrated by the fungous mycelium.

As indicated by the foregoing data those smut readings based on the visible hypertrophy and hyperplasia of infected tissue may be quite unreliable as far as

total smut infection is concerned. Plants which are to all appearances free from smut infection, may be infected at one or more points and may produce a visible smut gall only upon the chance occurrence of conditions favorable to gall production, or may in many cases never produce visible symptoms.

Fig. 9. Camera lucida drawing of smut mycelium in tissue of one of the floral primordia about midway between the base and the tip of a 65-day-old axillary bud removed from an inoculated sweet corn plant (Golden Bantam) 33 days after inoculation. X700.

THE EFFECT OF DATE OF PLANTING ON EXPRESSED NODAL INFECTION

To determine whether or not the date of planting affects the percentage of smut infection, plantings were made at weekly intervals and a final smut reading was made at the end of the growing season. Field corn (Reid's Yellow Dent) was planted in 1931, 1932 and 1933. The 1933 season presented such adverse conditions for late plantings that no data were obtained. The data for 2 years on date of planting as presented in table 12 and fig. 11 show that the percentage of corn plants which became naturally infected by smut as indicated by nodal smut galls is partially dependent upon the time of planting.

The increase in percentage of infection in late planted corn is probably due to the fact that weather conditions during the time the plants are 6 to 18 inches high are favorable for a more rapid succulent type of growth accompanied by more activity in the meristematic tissue than is present in earlier plantings when growth is somewhat retarded due to lower temperatures. The optimum temperature for growth of the corn smut organism has been found to be

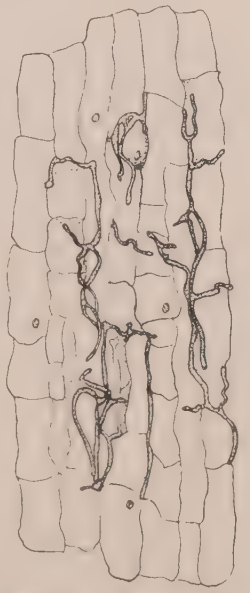

Fig. 10. Camera lucida drawing of smut mycelium in the tissue of a leaf or husk of an apparently disease-free 97-day-old axillary bud which was removed from an inoculated sweet corn plant (Golden Bantam) 67 days after inoculation. X700.

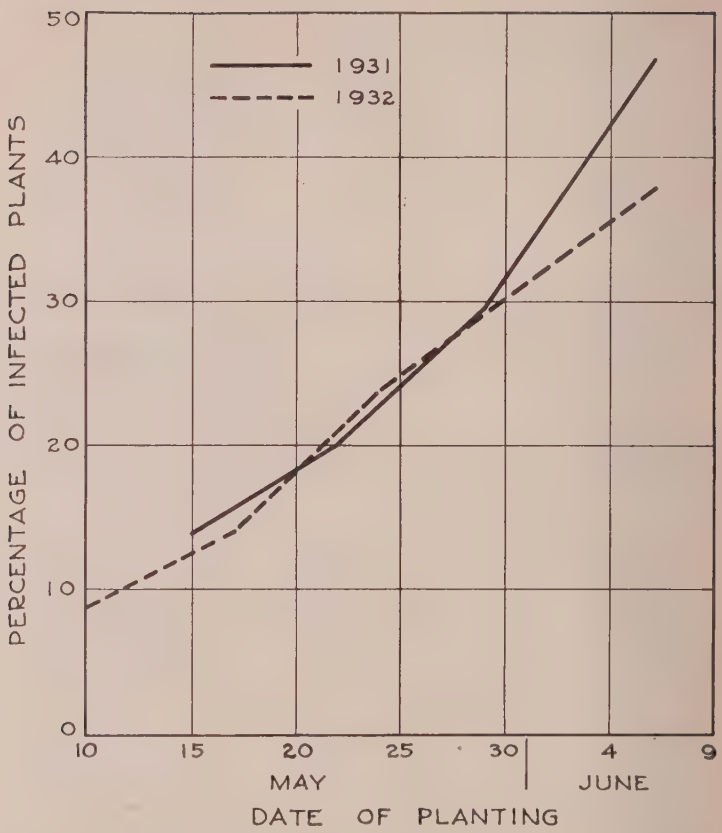

Fig. 11. Percentage of smut infection on field corn plants (Reid's Yellow Dent) for different dates of planting in 1931 and 1932.

TABLE 12. NUMBER AND PERCENTAGE OF PLANTS INFECTED WITH SMUT WITH DATE OF PLANTING IN 1931 AND 1932.

Date planted	Number plants	Number smutted	Percentage smutted
May 15, 1931	115	16	13.9
May 22, 1931	99	20	20.2
May 29, 1931	102	30	29.4
June 6, 1931	92	43	46.7
May 10, 1932	451	39	8.6
May 17, 1932	435	61	14.0
May 24, 1932	429	103	24.0
June 6, 1932	453	171	37.7

TABLE 13. PERCENTAGE OF SMUT EXPRESSION IN EIGHT RATES OF PLANTING, 1931, 1932, 1933 AND 1934.

Number plants per hill	1931		1932		1933		1934	
	Number plants	Percentage plants infected	Number plants	Percentage plants infected	Number plants	Percentage plants infected	Number plants	Percentage plants infected
1	80	40.8	137	20.4	69	9.0	105	22.8
2	159	23.2	266	13.9	132	10.6	230	11.2
3	235	22.5	520	12.5	165	4.3	273	14.6
4	300	29.0	524	15.4	204	5.4	408	17.6
5	370	32.4	710	21.6	220	5.9	470	18.5
6	452	30.7	828	20.0	258	8.9	472	17.5
7	520	41.1	413	24.7	——	——	579	17.4
8	585	34.7	.592	23.0	——	——	508	20.6

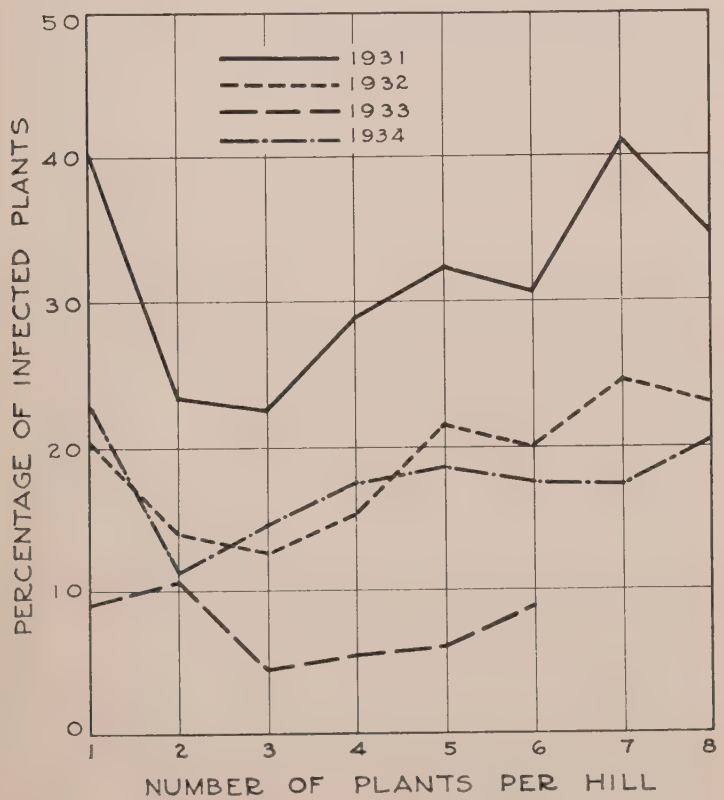

Fig. 12. Percentage of nodal smut expression in eight rates of planting (Reid's Yellow Dent) 1931, 1932, 1933 and 1934.

from 30° to 32° C. and it is possible that a greater supply of inoculum is present at later dates during periods of higher temperature.

EFFECT OF RATE OF PLANTING ON NODAL SMUT EXPRESSION

To determine the effect of the rate of planting on the percentage of plants naturally infected with corn smut, the hills were thinned to the desired number of plants 2 weeks after planting. Two rows of 79 hills each were thinned to one plant per hill, two rows were thinned to two plants per hill, etc., up to eight plants per hill. Each set of two rows was bordered by rows planted at the rate of three plants per hill. Field corn (Reid's Yellow Dent) was planted in 1931, 1932, 1933 and 1934.

The comparison of 4 years' results of rate of planting shown in table 13 and fig. 12 shows that while the amount of expressed infection varies from year to year there seems to be a tendency for the percentage of expressed infection to increase both ways from the two and three rates of planting.

DISCUSSION

In the past it has been assumed that the visible symptoms of corn smut represented the total amount of infection and injury to the corn plant. It has been clearly shown in this study, however, that the amount of corn smut infection is greater than the number of galls and leaf lesions indicates. It has been found that many infections in the nodal shoots remain covered by the leaf sheath. In data presented in connection with table 8 it was shown that an aggregate of 39.6 percent of the infections that develop far enough to become visible to the unaided eye remain hidden back of the leaf sheath. It is also evident from histological studies that there may be still other infections of the stem initials which never increase sufficiently to be seen with the unaided eye. It is probable that these infections would be expressed as smut galls if proper conditions existed. On the other hand, the possibility remains that the mycelium in such cases might be gametophytic and smut galls never would appear.

The significance of these smut infections that fail to produce visible galls has not been determined. Where the infection advances far enough to become visible the injurious effect on the plant can be measured in terms of yield, but where the infection fails to develop far enough to become visible the injury may be trifling. The presence of parasitic mycelium in the

ear and tassel initials, however, even though no gall develops, may have much to do with the size of the mature ear.

That the corn plant is most susceptible when about 1 foot high has been known since Brefeld's (2) excellent work in 1883, and has been substantiated by Clinton (5), Hitchcock and Norton (14) and Platz (23). This period of susceptibility of the corn plant coincides with its stage of development designated by Weatherwax (27) and Hershey (13) as the period of differentiation and comprises approximately the first 40 days of the plant's activity. The second phase, which covers the period from about 40 to 80 days, has to do with the elongation and enlargement of structures differentiated during the first period. Hershey (13) has shown that at about the beginning of the period of elongation the apical meristem is transformed into the primordia of the staminate inflorescence. He found that the formation of the tassel and cessation in the differentiation of axillary buds occurred simultaneously. Infection rarely occurs during this period of elongation as shown by Brefeld (2), Hitchcock and Norton (14) and Platz (23).

Platz (23) has also shown that nodal smut galls seldom appear on the corn plant until about 70 to 80 days after the date of planting, or some 40 days after the period of differentiation. In this event the smut organism in the axillary bud initials is evidently retarded in its development until approximately the termination of the period of elongation. This is shown by inoculation experiments in which no nodal galls appeared until 30 days after inoculation or 55 days after planting and by histological examination of axillary buds showing the presence of mycelium of the pathogene in buds which were in all appearances free from infection. In certain tissues other than the axillary buds, however, such as the staminate inflorescence or the region of indeterminate growth of the pistillate inflorescence, the smut organism apparently may continue to gall formation, which explains the appearance of the early expression of tassel and ear infection.

The period extending from about 80 days after date of planting until the death of the corn plant is utilized in the maturation of the axillary buds or ears. Morphological studies by Kudelka (21) and Weatherwax (28) have shown that as a rule every leaf axil has a bud of some kind. Below the ear on the main culm, these buds are usually prominent and in some varieties there may be a graded series from the main pistillate branch down through the smaller structures of the same kind to buds which have no function. During the period of maturation the smut pathogene may form a smut gall if located in an axillary bud, which resumes growth activity following the period

of elongation. On the other hand, if located in an axillary bud which persists in a latent condition, the pathogene may remain, as shown by histological examination of latent axillary buds, throughout the life of the corn plant without expressing its presence as a visible smut gall.

Kyle (22) believes the amount of ear infection to be influenced by the thickness of husk covering. Weatherwax (27) and Hershey (13) have shown, however, that while the growth of the tip of the ear is indeterminate, it seldom remains meristematic after the appearance of the silks, at which time it still is enclosed tightly by the husks. Platz (23) made numerous unsuccessful attempts to bring about ear infection by dropping a sporidial suspension behind the leaf sheaths. His only successful attempts, however, resulted from dropping a sporidial suspension with a pipette into the distal end of young ears. Similar infection experiments by the writer, with like results and the demonstration of mycelium in the ear initials, suggest that ear infection occurs before the ear has attained sufficient size to break its husk covering. It is thought that by the time the ear has attained sufficient size to be influenced by husk covering the exposed tissues are sufficiently mature to resist penetration by the mycelium of the pathogenes.

Data obtained on rate of planting have shown a tendency for expression of smut infection, as indicated by the presence of nodal smut galls, to increase both ways from the 2-3 rates of planting. A 4-year comparison of smut infection on plantings of three and five per hill gave approximately ten percent more smut galls on the fives than on the threes. The foregoing conclusions on time and expression of infection suggest that the larger number of smut galls per plant at rates of planting above or below three plants per hill is due to an increased expression of the disease rather than to an actual difference in the percentage of infection. Plants grown at different rates of planting unquestionably are subject to different environmental conditions, but from the preceding discussion it would appear likely that these conditions affect the physiological status of the host rather than the severity of infection. The period of greatest susceptibility for the plant is past before it is sufficiently large to affect its immediate surrounding.

Such a condition as that described in the foregoing discussion, in which the parasite is dependent to such an extent upon the proper development of the host for its expression, suggests the difficulty of differentiating a resistant plant from a susceptible plant in which for some reason expression has not appeared and the inadvisibility of selecting for resistance on the

basis of visible galls alone. It also emphasizes the desirability of the practice of selecting those lines of corn in which there is a predominant tendency to produce a single ear rather than several. While such lines might not be resistant, the possibilities for the production of smut galls would decrease as the number of active axillary buds decreased.

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